

Research Article

***In silico* analysis reveals the protective role of the *HBB* gene in cancers**Nabiha Tasneem Khan, Mustak Ibn Ayub^{1*} and Sabina Yeasmin^{1*}

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ABSTRACT

Hemoglobin subunit beta (HBB) plays a critical role in the oxygen-transporting function of red blood cells. Emerging evidence suggests that altered HBB expression is associated with the progression of several cancers, and reduced expression has been linked with poor prognosis in malignancies such as anaplastic thyroid cancer, ovarian cancer, and acute myeloid leukemia. In this study, we investigated the potential functional role of HBB in cancer using computational approaches. The analysis suggested a potential interaction between HBB and the tumor suppressor protein p53, suggesting their possible involvement in the folate metabolism pathway. Further analysis using the GEPIA and NCBI GEO databases indicated that the expression of HBB is lower in several cancerous tissues compared to their healthy counterparts. These analyses also showed that HBB shared similar expression patterns with the reported antitumor-associated proteins KLF6, BLCAP, BTNL9 and TBRG1 across squamous lung cancer, atypical ductal hyperplasia, ductal carcinoma *in situ*, mammary gland, and ovarian adenocarcinoma, respectively. Furthermore, the Kaplan-Meier plot from the Human Protein Atlas database showed better survival rates in kidney renal carcinoma, pancreatic cancer, and melanoma patients having higher levels of HBB expression. All these observations suggest that HBB may have protective effects against certain cancers.

Introduction

Hemoglobin subunit beta (HBB) is an essential component of hemoglobin protein in red blood cells that is responsible for transporting oxygen from the lungs to tissues. The HBB protein consists of 147 amino acids and possesses a molecular weight of 15,867 Da (Huang et al., 2022). The genetic code for HBB is located on human chromosome 11. Mutations in this gene can lead to abnormal hemoglobin variants that cause inherited blood disorders, including β -thalassemia and sickle cell disease (Galanello and Origa, 2010; Thein, 2013). Beyond its well-known role in hematological disorders, recent studies have reported altered HBB expression in several malignancies, suggesting a potential role in tumor biology. The expression of the *HBB* gene was found

to be significantly reduced in the gene expression profile of anaplastic thyroid cancer cell lines (ACL) (Onda et al., 2005). According to Yoshihara Ovarian Statistics data, *ADH1B*, *HBB*, *ABCA8*, and *HBA1* genes were 10-fold down-regulated in the samples of ovarian serous adenocarcinoma (Liu et al., 2015). A gene expression profiling exhibited that *HBB* expression was decreased 17 to 40-fold in lung metastatic lesions than in normal lung tissues (Rhodes et al., 2004). The expression of the *HBB* gene was notably reduced in individuals with acute myeloid leukemia (Luo et al., 2022). Increasing the expression of HBB may represent a potential strategy for suppressing K562 leukemia cell line proliferation, inducing programmed cell death, and inhibiting cell-

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cycle progression. This indicated that HBB may have the potential as a tumor suppressor in leukemia.

Previous studies had reported an association between HBB expression levels and the aggressiveness of breast cancer (Ponzetti et al., 2017). HBB-containing cells expressed higher transcriptional levels of vascular endothelial growth factor (VEGF) than HBB knockdown cells. Vascular endothelial growth factor promotes the formation of new blood vessels and, therefore, influences rapid tumor growth (Lunevicius et al., 2001; Radisky et al., 2007). Another study revealed that cervical cancerous tissue had significantly higher expression of the *HBA1* and *HBB* genes than healthy cervical tissues (Li et al., 2013). In addition, the genes including *EGR1*, *HBB*, *HBA1*, and *SPARC* were upregulated in astrocytomas (AA) and glioblastoma multiform (GBM) (Chang, 2008).

A short C terminal region (ENFRLGNNVLCVLA) of HBB termed Metox was discovered for the anti-tumor and anti-metastatic properties (Maman et al., 2017). Both human HBB and mouse HBB2 inhibited growth in lung-metastasizing neuroblastoma cells. Metox inhibited the growth of adrenal neuroblastoma tumors as well as lung and bone marrow invasiveness. However, the exact mechanism of HBB in tumor suppression has yet not been fully understood.

Dysregulated hemoglobin expression may contribute to increased reactive oxygen species (ROS) production in cancer cells. Elevated ROS levels can induce DNA damage and activate cellular stress response pathways, including the p53 signaling pathway (Aggarwal et al., 2019). Because HBB plays an important role in oxygen transport and cellular redox balance, alterations in its expression may influence intracellular ROS levels and subsequently affect p53-mediated regulatory mechanisms involved in cell cycle control and apoptosis. In this study, we have investigated the functional role of HBB in cancers using *in-silico* approaches.

Materials and Methods

Exploring the *HBB* gene expression

We explored The Human Protein Atlas database (<https://www.proteinatlas.org/>) for tissue specificity, immune cell specificity, and pathological status of

HBB (Uhlén et al., 2015). In our study, we used the GEPIA2 web software (<http://gepia2.cancer-pku.cn>) to investigate HBB expression across 33 human tumor types compared to normal matches.

HBB expression analysis with other associated genes in cancers

We searched the NCBI Gene Expression Omnibus (GEO) datasets for cancer-associated gene expression profiles that have similarities with *HBB* expression levels in different cancers in (Barrett et al., 2013). To identify relevant datasets, the GEO database was queried using the filters “Organism,” “Up/Down Gene Expression,” and “Cancer Dataset,” yielding 71 datasets that exhibited differential HBB expression between cancerous and normal tissues. Among these, four datasets representing squamous lung cancer, breast atypical ductal hyperplasia, ductal carcinoma in situ, and ovarian adenocarcinoma studies were selected for further analysis. Dataset selection was based on the presence of significant differential HBB expression, availability of matched normal control samples, and high-quality annotation with complete clinical and experimental metadata. Within each selected dataset, genes exhibiting expression patterns similar to HBB were identified using the GEO Profiles tool, which visualizes gene expression across all samples within a dataset.

HBB Protein Network

Based on literature review, HBB, MT1M, and TFPI2 were identified as risk biomarkers in breast cancer (Ma et al., 2020). Another study reported that genes including *HBB*, *ALDH1A2*, *ADH1B*, and *ABCA8* have differential expression in ovarian cancer (Liu et al., 2015). To investigate the potential functional associations of HBB with these proteins, protein-protein interaction (PPI) analysis was performed using the STRING database (version 12.0) (Szklarczyk et al., 2023). The interaction network was generated using a minimum required interaction score of 0.400 (medium confidence), and evidence was derived from the available STRING evidence channels, including experimental data, curated databases, co-

expression, gene neighborhood, gene fusion, gene co-occurrence, text mining, and co-expression.

HBB expression in Cancer Prognosis

Overall survival (OS) or Survival probability analysis was performed using The Human Protein Atlas by searching *HBB* gene expression and exploring the pathology section. Applying the default parameter Kaplan-Meier (KM) survival curves were generated for the *HBB* gene, with the best cutoff value and p-value <0.05. The study samples were categorized into high- and low-expression groups based on the median expression levels of *HBB*.

Result

Expression of the *HBB* gene is reduced in many cancer tissues

To investigate *HBB* protein expression across different tissues, we analyzed data from the Human Protein Atlas (HPA) database. Immunohistochemistry-based protein expression data revealed that *HBB* was highly enriched in the lung, placenta, and bone marrow. To further examine *HBB* gene expression in cancer, we analyzed transcriptomic data using the GEPIA2 platform. The bar plot (Fig. 1) shows the differential expression of *HBB* gene between tumor and corresponding normal tissues across multiple cancer types. The height of each bar represents the median expression level ($\log_2(\text{TPM} + 1)$) for each tumor or normal tissue. The analysis

demonstrated that *HBB* gene expression was reduced in most cancer tissues compared to normal tissues. Notably, *HBB* gene expression was significantly lower in lymphoid neoplasm diffuse large B-cell lymphoma (DLBC), lung squamous cell carcinoma (LUSC), and thymoma (THYM) compared to their corresponding normal tissues. These findings suggest that reduced *HBB* expression may be associated with tumor development and progression.

HBB had a similar expression pattern to other cancer-related genes

Analysis of GEO database profiles identified four independent cancer datasets, including squamous lung cancer, atypical ductal hyperplasia and breast cancer, ductal carcinoma in situ (DCIS) of the mammary gland, and ovarian carcinoma, which were used to evaluate the differential expression of the *HBB* gene between normal and cancer tissues (Fig. 2). Expression profiles of *HBB* and tumor suppressor genes *KLF6*, *BLCAP*, *BTNL9*, and *TBRG1* were analyzed using microarray datasets across various cancers. Red bars represent gene expression levels, and blue dots indicate percentile rank within samples. The genes we found from our analysis, all are related to suppressing tumor proliferation or inhibiting tumor growth. They had reduced expression in certain cancers than in normal tissues and the expression pattern of *HBB* closely resembled that of these established tumor suppressor genes.

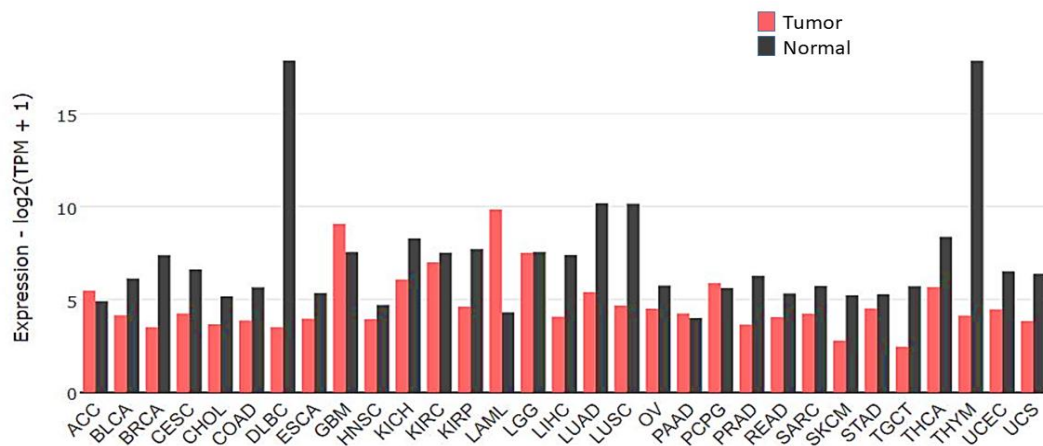


Fig. 1. Pan-cancer differential expression analysis of *HBB* gene expression in multiple cancer types where red bars indicate tumor tissues, while black bars indicate corresponding normal tissues. In most cancer types, *HBB* expression is lower in tumor tissues compared to normal tissues, suggesting downregulation of *HBB* gene expression during tumorigenesis.

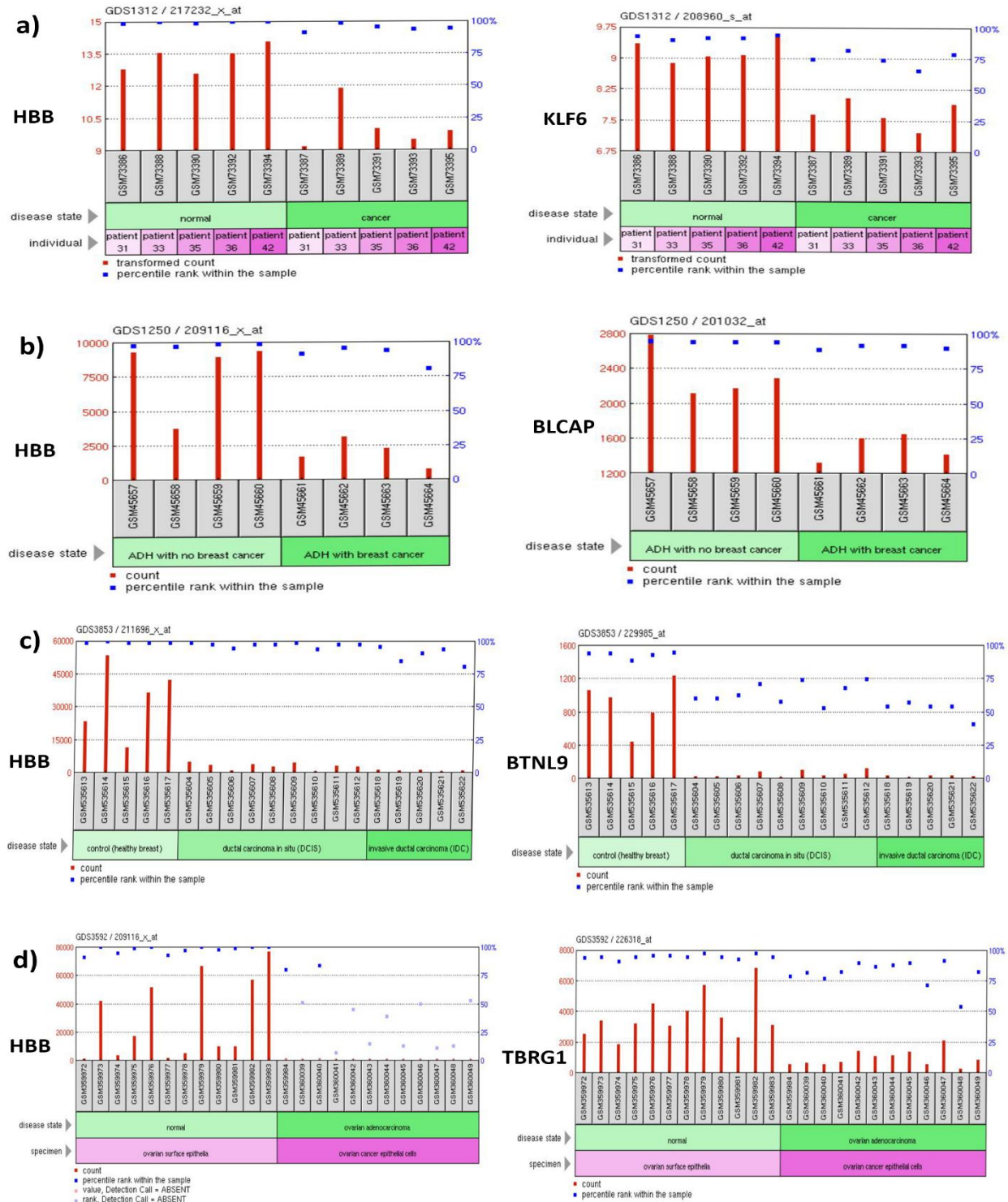


Fig. 2. Comparative expression analysis of *HBB* and tumor suppressor genes *KLF6*, *BLCAP*, *BTNL9*, and *TBRG1* using microarray datasets across various cancers. (a) Expression of *HBB* and *KLF6* in normal and squamous lung cancer samples. (b) Expression of *HBB* and *BLCAP* in atypical ductal hyperplasia with and without breast cancer. (c) Expression of *HBB* and *BTNL9* in normal breast tissue and invasive ductal carcinoma. (d) Expression of *HBB* and *TBRG1* in normal ovarian tissue and ovarian adenocarcinoma.

In squamous lung cancer, HBB had reduced expression similar to KLF6 (Kruppel-like factor 6). KLF6 is considered a tumor suppressor which inhibits cellular proliferation and tumor growth (Sabatino et al., 2019). In atypical ductal hyperplasia and breast cancer, the expression of BLCAP (bladder cancer-associated protein) was downregulated compared to normal tissues. BLCAP expression level was decreased in bladder cancer and the lower expression level was associated to poorer survival rates (Gromova et al., 2017). BLCAP functions as a tumor suppressor by promoting apoptosis and inhibiting cell proliferation. BTNL9 (butyrophilin-like 9) suppresses the invasion of uveal melanoma and inhibits proliferation and metastasis via the p53/CDC25C and p53/GADD45 pathways and exhibited reduced expression in breast cancer (Jiang and Liu, 2019). Similarly, both HBB and TBRG1 (Transforming growth factor beta regulator 1) had reduced expression in ovarian carcinoma. TBRG1 is known to suppress tumor growth by regulating p53 signaling pathway, inhibiting cell proliferation and maintaining chromosomal stability (Reed and Quelle, 2015).

Based on these findings, it can be inferred that HBB may play a protective role in certain cancers and may function as a potential tumor suppressor.

Protein-protein network analysis reveals the interaction between HBB and p53

From the literature review, HBB, MT1M, and TFPI2 were identified as potential risk biomarkers in breast cancer (Ma et al., 2020). TFPI2 (tissue factor pathway inhibitor 2) has been reported to be regulated by the tumor suppressor protein p53 (Yang et al., 2014). Downregulation of TFPI2 in breast cancer tissues is associated with poor patient prognosis, highlighting its tumor suppressive role (Zhao et al., 2020). STRING protein-protein interaction analysis revealed that TP53 interacts with HBB (Fig. 3). TP53 is a well-established tumor suppressor that regulates cell cycle progression, apoptosis, and genomic stability, thereby preventing uncontrolled cell proliferation (Reed and Quelle, 2015). The interaction between HBB and TP53 has been experimentally validated in studies investigating the folate metabolism pathway (Egoyenechea et al., 2024).

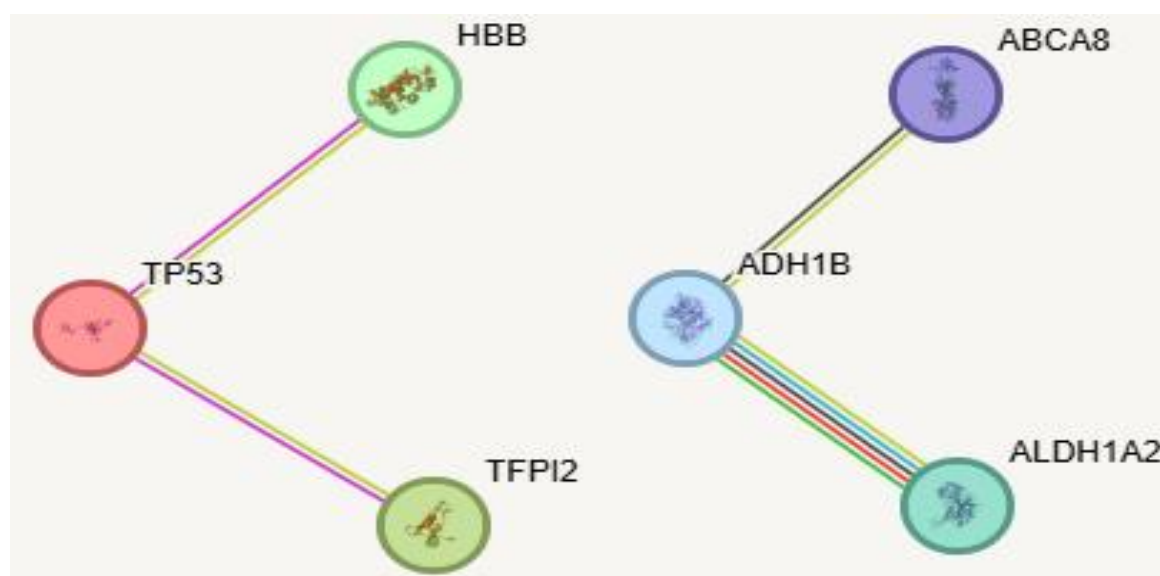


Fig. 3. Network of protein-protein interaction analysis of HBB protein with other cancer-associated proteins, including TP53, MT1M, ALDH1A2, ADH1B, and ABCA8. Nodes represent individual proteins, and edges represent protein-protein interactions. The interaction between HBB and TP53 is supported by experimental evidence, highlighting a potential functional association between HBB and tumor suppressor pathways.

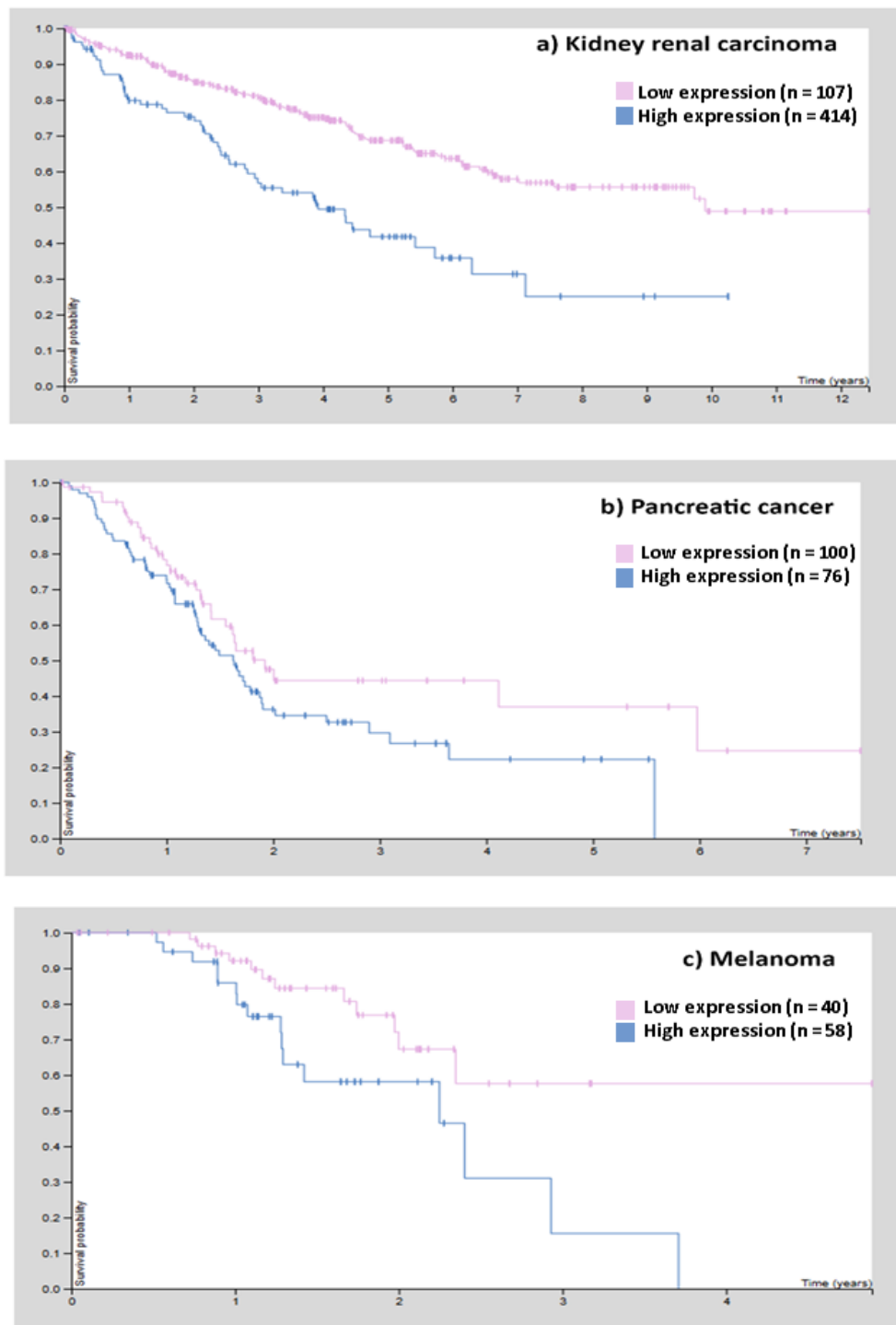


Fig. 4. Kaplan-Meier survival analysis comparing overall survival probability of patients stratified by high HBB expression (pink) and low HBB expression (blue) in (a) kidney renal carcinoma, (b) pancreatic cancer, and (c) melanoma. The x-axis represents time in years, and the y-axis represents survival probability. Patients with high HBB expression exhibit consistently higher survival probabilities compared to those with low HBB expression across all three cancer types.

Further analysis of the interaction network revealed that both HBB and TP53 are components of the broader folate metabolism and cellular stress response pathways. Although the exact molecular mechanism linking HBB and TP53 remains unclear, their association may be related to their roles in regulating oxidative stress. HBB is involved in oxygen transport and may contribute to reactive oxygen species (ROS) scavenging, while TP53 plays a critical role in cellular responses to oxidative damage. Therefore, we hypothesize that HBB may influence cancer progression through modulation of oxidative stress and interaction with TP53-regulated tumor suppressor pathways.

HBB expression is related to better survival in cancers

The survival probability between high HBB groups and low HBB groups was compared in different cancers. Fig. 4 shows Kaplan-Meier survival curves comparing overall survival between patients with high HBB expression (pink line) and low HBB expression (blue line) in three different cancers. The x-axis showed the period of years between the cancer diagnosis date and the patient's death and the y-axis was used to indicate the survival possibility. The graph interpreted that patients with high HBB expression had significantly higher survival probability compared to low HBB expression in kidney renal carcinoma, pancreatic cancer, and melanoma. The slower decline in survival curves in the high HBB group indicated that HBB expression is associated with favorable prognosis and may serve as a positive prognostic biomarker in multiple cancers.

Discussion

Variants in the *HBB* gene that result in structurally abnormal or nonfunctional beta hemoglobin can lead to various hemoglobinopathies. Furthermore, multiple studies have discovered that beta hemoglobin gene expression is reduced in a variety of cancers (Onda et al., 2005; Liu et al., 2015; Luo et al., 2022). In this study, we have investigated the prospective protective role of HBB against cancers using different computational analyses. We started with the finding of reduced expression of

HBB in cancers than in normal tissues. We further explored the expression profile of established anti-tumor genes and observed similar expression to that of *HBB* across specific cancers. These findings suggest a potential protective role for HBB in cancer.

We assume that the protective role of HBB against certain cancers might be related to its interaction with p53, a well-known tumor suppressor protein. Interestingly, tandem mass spectrometry identified hemoglobin as a potential p53-interacting protein in human placental and liver tissues (Kikuchi et al., 2003).

Our analysis has found the presence of both HBB and p53 in the complex folate metabolism pathway (Egoyenechea et al., 2024). It is still unclear how folate and HBB might be involved in p53 regulation. However, both HBB and folate independently function as a scavenger of reactive oxygen species (ROS) which can regulate p53 activation (Aggarwal et al., 2019; Li et al., 2022). Based on these observations, we propose a potential tumor-suppressive mechanism of HBB involving the regulation of cellular redox homeostasis and p53 signaling. We hypothesize that reduced HBB expression may compromise ROS regulation, resulting in oxidative stress that could influence p53 activity and cancer-associated cellular processes. Under certain conditions, moderate ROS accumulation may contribute to tumor-promoting cellular environments, whereas excessive ROS accumulation may activate p53-mediated cell-cycle apoptosis. Thus, HBB may potentially contribute to cancer suppression by maintaining ROS homeostasis and influencing p53-dependent cellular responses. However, this proposed mechanism remains hypothetical and requires experimental validation to establish the functional relationship between HBB expression, ROS accumulation, and p53 activation. Future in vitro and in vivo studies are required to confirm the functional role of HBB in folate metabolism, oxidative stress regulation, and TP53-mediated tumor suppression.

Moreover, our analyses have identified that in most of the cases, higher HBB expression might be related to

better survival in cancers. Overall, our *in silico* study suggests a protective role of HBB against cancers, and while the association between HBB and p53 in cancer is still an area of ongoing research, our work indicates a probable role of HBB in regulating the apoptotic function of p53 through the folate metabolism pathway. Further research is necessary to confirm the findings and assumptions of this study.

Statements and declarations

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Authors contribution

Mustak Ibn Ayub and Sabina Yeasmin: Conceptualization and study design. Nabihha Tasneem Khan: Material preparation, data collection, and analysis. Nabihha Tasneem Khan: Written the first draft. All authors commented on drafted versions of the manuscript. All authors read, reviewed, and approved the final manuscript.

Conflict of interest

The authors have no relevant financial or non-financial interests to disclose.

Data and software availability

All figures are provided. In this research work publicly available free online software/tools were used. Necessary link and reference of the software/tools provided in the method section.

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